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Effects of glutathione *S*-transferase A1 (*GSTA1*) genotype and potential modifiers on breast cancer risk

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Glutathione *S*-transferases (GSTs) are phase II enzymes that are involved in the detoxification of a wide range of carcinogens. The novel *GSTA1A* and *GSTA1**B* genetic polymorphism results in differential expression, with lower transcriptional activation of *GSTA1**B* (variant) than that of *GSTA1**A* (common) allele. Considering that cruciferous vegetables induce GSTs, which metabolize tobacco smoke carcinogens, we hypothesized that the variant *GSTA1**B* genotype may predispose women to breast cancer, particularly among low cruciferous vegetable consumers and among smokers. Thus, we evaluated potential relationships between *GSTA1* polymorphisms and breast cancer risk, in relation to vegetable consumption and smoking status in the Long Island Breast Cancer Study Project (1996–1997), a population-based case–control study. Genotyping (1036 cases and 1089 controls) was performed, and putative breast cancer risk factors and usual dietary intakes were assessed. Having *GSTA1**A/**B* or **B/**B* genotypes was not associated with increased breast cancer risk, compared to having the common **A/**A* genotype. However, among women in the lowest two tertiles of cruciferous vegetable consumption, **B/**B* genotypes were associated with increased risk (OR (95% CI) = 1.73 (1.10–2.72) for 0–1 servings/week), compared to women with **A/**A* genotypes. Among women with **B/**B* genotypes, a significant inverse trend between cruciferous vegetable consumption and breast cancer risk was observed (*P* for trend = 0.05), and higher consumption (4+ servings/week) ameliorated the increased risk associated with the genotype. Current smokers with **B/**B* genotypes had a 1.89-fold increase in risk (OR (95% CI) = 1.89 (1.09–3.25)), compared with never smokers with **A/**A* genotypes. These data indicate that *GSTA1* genotypes related to reduced *GSTA1* expression are associated with increased breast cancer primarily among women with lower consumption of cruciferous vegetables and among current smokers.**

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; *GSTA1*, Glutathione *S*-transferase A1; HWE, Hardy–Weinberg Equilibrium; ITC, isothiocyanates; LIBCSP, Long Island Breast Cancer Study Project; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; OR, odds ratio; VIF, variance inflation factor.

Introduction

Glutathione *S*-transferases (GSTs) are a family of phase II enzymes that are involved in the detoxification of carcinogens, environmental toxins and products of oxidative stress, by catalyzing conjugation with glutathione (1,2). GST alpha class is the primary hepatic GST, but is also expressed in human breast (2). Preferred substrates of GSTA1 include polycyclic aromatic hydrocarbons (e.g. benzo[*a*]pyrene), known tobacco smoke carcinogens (3). The GSTs, including GSTA1, are also involved, to some extent, in the metabolism of isothiocyanates (ITCs), potent anti-carcinogens that are derived from consumption of cruciferous vegetables (4), including broccoli, cabbage, cauliflower, Brussels sprouts, kale and collard greens. Notably, GSTs are also induced by these dietary components.

GSTA1 may be relevant for breast cancer through metabolism of carcinogens, such as those found in tobacco smoke, or through relationships with ITCs. There is some indication from epidemiological studies that cruciferous vegetable consumption is associated with reduced breast cancer risk, but results are not consistent (5–7). No associations were observed in the Long Island Breast Cancer Study Project (LIBCSP), upon which these analyses are based [OR (95% CI) = 1.00 (0.75–1.37)] (8). There are indications in the literature that relationships between cruciferous vegetables and cancer risk could be modified by variability in the GSTs.

A potential role for tobacco smoke carcinogens in breast cancer etiology is controversial. Experimental data indicate that cigarette smoke contains potential human breast carcinogens (9), and that these carcinogens reach the breast and can interact with cellular DNA (10–12). However, a meta-analysis evaluating active smoking and breast cancer risk showed only limited evidence for such an association (10). In the LIBCSP, there were also no significant associations observed between smoking and breast cancer risk [OR (95% CI) = 1.06 (0.76–1.48) for active current smokers; 1.33 (0.97–1.83) for pack years >20] (13). Because smoke contains numerous carcinogens and also appears to be anti-estrogenic, it is possible that associations only exist among subsets of women based upon metabolic variability, such as those with lower detoxification due to variants in phase II enzymes.

The novel *GSTA1*A* and *GSTA1*B* genetic polymorphism, containing three linked base substitutions in the promoter at positions −567, −69 and −52, results in differential expression (14), with lower transcriptional activation with *GSTA1*B* (variant) than with *GSTA1*A* (common) alleles *in vitro* (15). Individuals with *GSTA1*A* have T, C and G at positions −567, −69 and −52, respectively, and those with *GSTA1*B* have G, T and A (3). In a directed mutagenesis assay, the G→A change at position −52 (NCBI rs no. 3957356) was responsible for differential promoter activity, and this modification also altered binding of the ubiquitous transcription factor SP1 (15). We (C.B.A.) previously reported differences in breast cancer survival after therapy associated with this *GSTA1* genotype (16). To our knowledge, the associations between the novel *GSTA1* genotype and breast cancer risk have not been evaluated and, therefore, merit further investigation.

Considering that *GSTA1* polymorphisms may affect the detoxification efficiency of carcinogens, and that cruciferous vegetable consumption and cigarette smoking are sources of exposure to anti-carcinogens and carcinogens respectively, we hypothesized that the variant *GSTA1*B* genotype may predispose women to breast cancer, particularly those who are low vegetable consumers or smokers. Thus, we evaluated whether *GSTA1*B* genotype was associated with increased breast cancer risk, and whether the association between *GSTA1*B* genotype and breast cancer were higher among lower vegetable consumers or smokers in the LIBCSP.

Materials and methods

Study population

The LIBCSP, a population-based case–control study of breast cancer, was described previously (17). In brief, the cases were English-speaking women >20 years of age with newly diagnosed, primary *in situ* or invasive breast cancer who resided in Nassau and Suffolk Counties in Long Island, New York. Incident cases were ascertained between 1 August 1996, and 31 July 1997, using a rapid reporting network, developed by the study investigators. English-speaking controls, who were residents of the same two counties as the cases but did not have a history of breast cancer, were identified using Waksberg's method of random-digit dialing (RDD) (18) for women under the age of 65 years, and from Health Care Finance Administration (HCFA) rosters for women who were 65 years or older. Controls were frequency matched to the expected age distribution of case women by 5-year age groups. All respondents signed informed consent forms prior to the study interview.

Upon receiving physician and participant consent, 1508 cases (82.1%) and 1556 controls (62.8%) were interviewed in their homes by a trained interviewer. Among case and control respondents who completed the interviewer-administered questionnaire, 98.2 and 97.6% self-completed the food frequency questionnaire (FFQ), and 73.0 and 73.3% donated a blood sample (17). As previously published (17), an increase in breast cancer among women on Long Island was found to be associated with lower parity, late age at first birth, little or no breastfeeding, a family history of breast cancer, and increasing income and education. Results were similar when analyses were restricted to respondents who donated blood (17), or for those for whom DNA samples were available (data not shown). Cigarette smokers were less likely to donate a blood sample (17), but case–control status and fruit and vegetable consumption were not predictors of blood donation (data not shown).

Measurements

GSTA1 genotyping. Genomic DNA was extracted from mononuclear cells in whole blood separated by Ficoll (Sigma Chemical, St Louis, MO) and washed twice with phosphate-buffered saline (PBS). Pelleted cells were frozen at −80°C until DNA was isolated from them by standard phenol and chloroform isoamyl alcohol extraction and RNase treatment (19). Genotyping was performed by BioServe Biotechnologies (Laurel, MD) using Sequenom's high-throughput matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (20). PCR was performed in a total volume of 5 μl, containing 10× Buffer B (Solis Biodyne), 0.5 μl; DNA (diluted to 2.5 ng/μl), 2 μl; primers (5′-ACGTTGGATGTTAAACGCTGTCACCGTC CT-3′ and 5′-ACGTTGGATGGAGTGGCTTTTCCCTAACTTG-3′), at 2 pmol/μl and 0.5 μl each; $MgCl_2$ (25 mM, 0.5 μl); dNTPs (2.5 mM each, 0.25 μl); *Taq* polymerase (Qiagen), 0.02 μl; and water 0.73 μl.

All genotyping results were reviewed manually for quality control. Controls for genotype and two 'no template' controls were included on each plate. In addition, 170 sets of blinded controls (8%) were distributed throughout the plates for quality control purposes. There was excellent observer agreement in the 8% of randomly selected duplicates of genotyping results that were included for quality control purposes (kappa statistic of 0.92) with <1% failure rate of the assay. Laboratory personnel were blinded to case–control status. *GSTA1* genotype data were available for 1036 women with breast cancer and 1089 population-based controls.

Other exposure assessment. The LIBCSP questionnaire focused on known and suspected risk factors for breast cancer, including reproductive, hormonal, medical and lifestyle histories. To assess individual diets for the 12 months prior to interview, 98.2% of cases and 97.6% control participants completed a self-administered modified NCI-Block FFQ that was previously validated (21). Frequency and portion size of each food item were converted to a common denominator of 0.5 cup servings per week. Vegetable consumption was categorized into four groups: total vegetables, cruciferous vegetables, yellow vegetables and leafy vegetables, as previously described (8). Coleslaw, cabbage, sauerkraut, broccoli, cauliflower, Brussels sprouts, mustard greens, turnip greens, collards and kale comprised the cruciferous vegetable category. Carrots, yams, sweet potatoes and winter squash were categorized into yellow vegetables. Spinach, green salad, mustard greens, turnip greens, collards and kale were categorized into leafy vegetables. Consumptions of total and specific vegetables (cruciferous, yellow and leafy) were divided by tertiles, based on the distributions of controls for each factor.

A current cigarette smoker was defined as a smoker within the 12 months prior to the reference date (defined as date of diagnosis for cases and date of identification for controls); a former smoker was defined as a smoker who reported quitting >12 months prior to the reference date, as previously described (13). Pack-years and duration were categorized by median values of pack-years of smoking, among current and former smokers: pack-years (16.35 pack-years).

Statistical analysis

Unconditional logistic regression (22) was used to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI) for breast cancer, in relation to genotype. Tests for Hardy–Weinberg equilibrium (HWE) among controls were conducted using observed genotype frequencies and a χ^2-test with one degree of freedom. Risk associated with the variant **B/*B* or **A/*B* genotypes was computed in reference to the common **A/*A* genotype.

Final multivariate models were adjusted simultaneously for age at reference date. Factors tested but found not to confound the associations of interest included: menopausal status, active smoking status, number of pregnancies, age at first pregnancy, history of benign breast disease, fertility problems, age at menarche, hormone replacement therapy, family history, body mass index and lifetime alcohol intake. The final multivariate-adjusted models shown include those factors that either changed the estimated effect by 10% or more, or that remained in a best-fitting model, which was developed by starting with a full model and then excluding covariates that did not improve the overall fit, as measured by the −2log-likelihood ratio test (22).

Gene–vegetable consumption interactions were evaluated by joint categories of *GSTA1* genotype and vegetable intake. For vegetable consumption data, participants with daily energy intakes >3500 kcal or <400 kcal (cases = 36 and controls = 42) were dropped from the analyses. Total caloric intake was included in the multivariate model to control for confounding by total energy intake (23). In the fully adjusted model, we further adjusted associations with each category by the other specific categories of vegetables. For example, models assessing cruciferous vegetables were adjusted for yellow, leafy and total vegetables. Because there is concern about multi-colinearity when vegetable variables are adjusted together in the fully adjusted model, multi-colinearity was tested by calculating variance inflation factor (VIF) (24). However, because the fully adjusted model did not substantially change the estimates of effect, only the age- and total calorie-adjusted results are shown in the Table II.

Gene–smoking interactions were evaluated by joint categories of *GSTA1* genotype and smoking (i.e. active smoking status and pack years of smoking). To test statistical interactions on a multiplicative scale, a cross-product term of the ordinal score for each genotype and the risk factor variables (e.g. genotype × vegetables or smoking) was included in multivariate models. The log-likelihood statistic for models that included a multiplicative interaction term was compared to those that did not. Tests for trend were conducted using the ordinal values for *GSTA1* genotype.

Results

Among those with DNA available, 94% of cases and 93% of controls were Caucasian; 4% of cases and 4% of controls were African-Americans. Age range of cases and controls were 25.1–98.1 years (mean = 58.7, median = 57.8) and 20.3–95.5 years (mean = 56.1, median = 55.6), respectively. Thirty two percent of cases and 34% of controls were pre-menopausal women. Among case women with information on hormone receptor status and *GSTA1* genotype (n = 667), 407 (61.0%) were diagnosed with an ER+PR+ tumor, 95 (14.2%) had an ER+PR– tumor, 31 (4.7%) had an ER–PR+ tumor and 134 (20.1%) had an ER–PR– tumor. Overall median and interquartile distribution of total fruit and vegetable consumption were 14 (range, 9–21) servings per week for cases and 15 (range, 10–22) servings per week for controls. Current smokers often tended to smoke more than former smokers in both mean duration and pack years (35.6 ± 11.7 years and 32.7 ± 29.8 pack years per day for current smokers, and 21.2 ± 13.7 years and 18.7 ± 23.5 for former smokers). In these data, there were 739 former smokers (34.4% of cases and 35.8% of controls) with a mean of 19.05 ± 12.45 (range, 1.08–62.64) years since quitting smoking.

GSTA1 genotypes and breast cancer risk

Associations between *GSTA1* genotypes and breast cancer risk are shown in Table I. **A/*A*, **A/*B*, and **B/*B* genotypes were present in 35, 48 and 17% of controls, and the genotype distribution followed HWE ($P = 0.84$) among controls. **A/*B* and **B/*B* genotypes were not associated with breast cancer risk. Risk associated with **B/*B* genotypes was slightly higher in pre-menopausal women; however, there was no statistical interaction by menopausal status ($P = 0.44$).

GSTA1 genotypes, vegetable consumption and breast cancer risk

The ORs for breast cancer risk by *GSTA1* genotypes and vegetable consumption are shown in Table II. We observed a significant 70% increase in risk among women with the **B/*B* genotypes and the lowest two tertiles of consumption of cruciferous vegetables [OR (95% CI) = 1.73 (1.10–2.72) for 0–1 servings/week (tertile 1); OR (95% CI) = 1.77 (1.15–2.77) for 2–3 servings/week (tertile 2)], compared with those with **A/*A* genotypes and the lowest tertiles of intake. These estimates changed little when we adjusted for other vegetables in the multivariate model [data not shown, further adjusted yellow, leafy, and total vegetables, OR (95% CI) = 1.72 (1.10–2.72) for 0–1 servings/week (tertile 1); OR (95% CI) = 1.84 (1.18–2.88) for 2–3 servings/week (tertile 2)].

Although the **B/*B* homozygotes comprised only 17% of the population (controls), a significant inverse trend between cruciferous vegetable consumption and breast cancer risk was observed in these women (P for trend = 0.05). This trend was not detected among women with the **A/*A* or **A/*B* genotypes (P for trend = 0.35 and 0.94, respectively). Among women with **B/*B* genotypes, higher cruciferous vegetable (4+ servings/week, tertile 3) consumption ameliorated the expected increased risk associated with the **B/*B* genotypes. In an analysis restricted to the **B/*B* genotypes, the OR for the highest tertile (4+ servings/week) versus the lowest tertile of cruciferous vegetable consumption (0–1 serving/week) was 0.57 (95% CI, 0.33–0.98). Conversely, associations between *GSTA1* genotypes and breast cancer risk were most pronounced among women whose consumption was in the lowest tertile of cruciferous vegetables (P for trend = 0.01). However, statistically significant associations between *GSTA1* **B/*B* genotype and risk were not detected among women in the third tertile of cruciferous vegetable consumption [**B/*B* genotype and 4+ servings/week, OR (95% CI) = 1.00 (0.62–1.62), P for trend = 0.89]. Multiplicative interactions between *GSTA1* genotypes and cruciferous vegetable consumption in relation to breast cancer risk were not statistically significant (P for multiplicative interaction = 0.10).

Lower consumption of yellow or leafy vegetables with **B/*B* genotypes was not associated with an increased risk of breast cancer. We observed a 54% increase in risk (OR (95% CI) = 1.54 (1.08–2.38)) among the lowest consumers of total vegetables with **B/*B* genotypes. However, this association was not significant and somewhat attenuated when we adjusted for other vegetables, including cruciferous vegetables [OR (95% CI) = 1.44 (0.93–2.23)]. When we tested for multi-colinearity among the vegetable intake variables by calculating the VIF, the values were <10; thus the vegetable categories were not colinear (cruciferous vegetables: 1.05, yellow vegetables: 1.59, leafy vegetables: 1.79 and total vegetables: 2.65). Inverse trends between breast cancer risk and yellow, leafy, and total vegetable consumption were not statistically significant among women with **B/*B* genotypes, as well as **A/*B* and **A/*A* genotypes (data not shown).

The associations among *GSTA1*, vegetable consumption and breast cancer risk did not differ by menopausal status. Associations between risk and **B/*B* genotypes among the lowest tertile of consumers were similar for pre-menopausal [OR (95% CI) = 1.66 (0.88–3.11)] and post-menopausal [OR (95% CI) = 1.56 (0.91–2.71)] women, although cell sizes were small and estimates somewhat unstable.

GSTA1 genotypes, smoking status and breast cancer risk

Current smokers with *GSTA1*B/*B* genotypes had an 89% increase in breast cancer risk [OR (95% CI) = 1.89 (1.09–3.25)], compared to those with **A/*A* genotypes who never smoked (Table III). The joint effects of smoking and *GSTA1* genotypes in relation to breast cancer risk were somewhat more pronounced among pre-menopausal women. Current

Table I. Breast cancer risk associated with *GSTA1* polymorphisms: Long Island Breast Cancer Study Project, 1996–1997

	Cases	(%)	Controls	(%)	OR[a]	95% CI
Total participants	1036		1089			
**A/*A*	342	33	386	35	1.00	
**A/*B*	498	48	522	48	1.08	0.89–1.30
**B/*B*	196	19	181	17	1.20	0.94–1.54
Pre-menopausal women[b]	331		367			
**A/*A*	110	33	135	37	1.00	
**A/*B*	153	46	179	49	1.04	0.75–1.46
**B/*B*	68	21	53	14	1.46	0.94–2.28
Post-menopausal women[b]	682		677			
**A/*A*	223	33	229	34	1.00	
**A/*B*	332	49	324	48	1.07	0.84–1.36
**B/*B*	127	19	124	18	1.06	0.78–1.45

[a]Unconditional logistic regression adjusted for age.
[b]Excluding 23 cases and 45 controls with missing information on menopausal status.
*P for multiplicative interaction by menopausal status = 0.44.

Table II. Breast cancer risk associated with *GSTA1* polymorphisms by consumption of vegetables: Long Island Breast Cancer Study Project, 1996–1997

	Low vegetable intake (tertile 1)[a]			Intermediate vegetable intake (tertile 2)[a]			High vegetable intake (tertile 3)[a]		
	Cases[c]	Controls[c]	OR(95% CI)[b]	Cases[c]	Controls[c]	OR(95% CI)[b]	Cases[c]	Controls[c]	OR(95% CI)[b]
Cruciferous vegetable	0–1 servings/week			2–3 servings/week			4+ servings/week		
	299	327		370	352		315	355	
*A/*A	87	122	1.00 (ref)	127	117	1.54 (1.05–2.24)	107	119	1.23 (0.84–1.81)
*A/*B	144	151	1.31 (0.92–1.88)	167	177	1.33 (0.94–1.88)	163	175	1.30 (0.92–1.85)
*B/*B	68	54	1.73 (1.10–2.72)	76	58	1.77 (1.15–2.77)	45	61	1.00 (0.62–1.62)
Yellow vegetable	0–1 servings/week			2–3 servings/week			4+ servings/week		
	325	330		330	351		332	354	
*A/*A	99	118	1.00 (ref)	114	127	1.00 (0.69–1.46)	111	114	1.08 (0.73–1.57)
*A/*B	164	155	1.22 (0.86–1.73)	143	166	0.97 (0.68–1.37)	167	182	1.03 (0.73–1.45)
*B/*B	62	57	1.30 (0.80–1.98)	73	58	1.39 (0.90–2.17)	54	58	0.99 (0.62–1.57)
Leafy vegetable	0–2 servings/week			3–6 servings/week			7+ servings/week		
	349	352		352	364		286	319	
*A/*A	115	132	1.00 (ref)	112	125	1.03 (0.72–1.47)	97	102	1.07 (0.73–1.56)
*A/*B	157	161	1.10 (0.79–1.54)	168	184	1.05 (0.76–1.46)	149	158	1.06 (0.75–1.49)
*B/*B	77	59	1.43 (0.93–2.18)	72	55	1.46 (0.94–2.25)	40	59	0.87 (0.48–1.24)
All vegetable	0–11 servings/week			12–19 servings/week			20+ servings/week		
	328	350		377	343		283	342	
*A/*A	106	130	1.00 (ref)	112	120	1.11 (0.77–1.60)	106	109	1.15 (0.79–1.68)
*A/*B	152	162	1.12 (0.80–1.58)	187	163	1.38 (0.99–1.93)	136	178	0.90 (0.64–1.28)
*B/*B	70	58	1.54 (1.08–2.38)	78	60	1.31 (0.84–2.05)	41	55	0.83 (0.51–1.37)

[a]Vegetable consumption based on tertiles (low, intermediate, high, respectively) of control group.
[b]ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age and total calorie.
[c]Excluding 52 cases and 55 controls with missing or unreliable (daily total caloric intake <400 kcal or > 3500) information on diet.
*P for multiplicative interaction: 0.10 (cruciferous vegetable), 0.50 (yellow vegetable), 0.19 (leafy vegetable) and 0.17 (total vegetable).

Table III. Breast cancer risk associated with *GSTA1* polymorphisms by smoking status: Long Island Breast Cancer Study Project, 1996–1997

	Cases[a]	Controls[a]	OR (95% CI)[b]	Cases[a]	Controls[a]	OR (95% CI)[b]	Cases[a]	Controls[a]	OR (95% CI)[d]
Total participants									
Active smoking status[c]	Never smokers			Former smokers			Current smokers		
*A/*A	145	167	1.00 (ref)	125	132	1.06 (0.76–1.48)	69	81	1.05 (0.75–1.68)
*A/*B	238	249	1.09 (0.82–1.45)	171	177	1.10 (0.81–1.50)	85	91	1.15 (0.79–1.67)
*B/*B	96	78	1.39 (0.95–2.02)	57	77	0.82 (0.55–1.24)	40	26	1.89 (1.09–3.25)
Pack years[d]	Never smokers			Pack years <16.35			Pack years ≥ 16.35		
*A/*A	145	167	1.00 (ref)	71	91	0.91 (0.63–1.31)	122	121	1.20 (0.85–1.71)
*A/*B	238	250	1.09 (0.82–1.45)	105	117	1.07 (0.77–1.48)	149	148	1.18 (0.85–1.66)
*B/*B	96	78	1.39 (0.95–2.02)	34	50	0.83 (0.53–1.31)	62	51	1.45 (0.92–2.31)
Pre-menopausal women									
Active smoking status[c]	Never smokers			Former smokers			Current smokers		
*A/*A	55	61	1.00 (ref)	32	39	0.82 (0.45–1.50)	21	35	0.70 (0.36–1.35)
*A/*B	70	91	0.84 (0.52–1.37)	48	54	0.91 (0.53–1.57)	33	32	1.15 (0.67–2.12)
*B/*B	32	30	1.07 (0.57–2.00)	15	15	1.01 (0.45–2.28)	21	8	2.69 (1.09–6.63)
Pack years[d]	Never smokers			Pack years <16.35			Pack years ≥ 16.35		
*A/*A	55	61	1.00 (ref)	30	45	0.73 (0.41–1.33)	23	28	0.84 (0.43–1.64)
*A/*B	70	92	0.83 (0.51–1.35)	46	55	0.91 (0.53–1.57)	33	29	1.17 (0.63–2.18)
*B/*B	32	30	1.07 (0.57–2.00)	18	17	1.11 (0.51–2.38)	18	5	3.47 (1.20–10.89)
Post-menopausal women									
Active smoking status[c]	Never smokers			Former smokers			Current smokers		
*A/*A	88	97	1.00 (ref)	88	86	1.18 (0.78–1.79)	47	40	1.48 (0.88–2.49)
*A/*B	162	151	1.21 (0.84–1.75)	118	114	1.22 (0.82–1.80)	50	56	1.15 (0.71–1.88)
*B/*B	63	46	1.51 (0.94–2.45)	42	60	0.83 (0.51–1.36)	19	18	1.34 (0.66–2.74)
Pack years[d]	Never smokers			Pack years <16.35			Pack years ≥ 16.35		
*A/*A	88	97	1.00 (ref)	49	56	1.02 (0.63–1.65)	85	70	1.47 (0.95–2.27)
*A/*B	162	151	1.21 (0.84–1.75)	80	80	1.23 (0.80–1.89)	88	89	1.19 (0.78–1.80)
*B/*B	63	46	1.51 (0.94–2.45)	24	40	0.72 (0.40–1.29)	36	37	1.19 (0.69–2.06)

[a]Excluding 10 cases and 11 controls with missing information on smoking.
[b]ORs and 95% CIs calculated by unconditional logistic regression, adjusted for age.
[c]Active smoking status: a current cigarette smoker was defined as a smoker within the 12 months prior to the reference date (defined as date of diagnosis for cases and date of identification for controls); a former smoker was defined as a smoker who reported quitting >12 months prior to the reference date.
[d]Pack years was divided by on median of among current or former smokers of controls (16.35 pack years).
*P for multiplicative interaction: 0.12 (active smoking, total participants), 0.51 (packyears, total participants), 0.25 (active smoking, pre-menopausal women), 0.32 (packyears, pre-menopausal women), 0.18 (active smoking, post-menopausal women), and 0.14 (packyears, post-menopausal women).

pre-menopausal smokers with the *GSTA1*B/*B* genotypes had a 2.69-fold increase in the risk of getting breast cancer [OR (95% CI) = 2.69 (1.09–6.63)], compared with those with **A/*A* genotypes, who never smoked. We stratified by dose (pack-years of smoking, median). Heavier smokers (≥16.35 pack-years) with **B/*B* genotypes, experienced the most pronounced increase in their risk of breast cancer, compared to lighter smokers (<16.35 pack-years) or nonsmokers with **A/*A* genotypes. Yet, cell sizes were small, and risk estimates were unstable (Table III). Among post-menopausal women, smoking status did not modify the association between the *GSTA1* genotype and breast cancer risk, although women with **B/*B* genotypes who had never smoked had a somewhat increased risk of breast cancer.

Discussion

In this large population-based study, we found that associations between a polymorphism adversely affecting expression of *GSTA1* and breast cancer risk were primarily observed among women in the lowest two tertiles of cruciferous vegetable consumption, and among smokers. A significant inverse trend was observed between cruciferous vegetable consumption and breast cancer risk (P for trend = 0.05) among women with the **B/*B* genotype. Higher consumption (4+ servings/week) ameliorated the observed increased risk associated with the genotype. This finding is consistent with the hypothesis that *GSTA1* polymorphisms may increase breast cancer risk in environments with significant exposure to carcinogens (cigarette smoke), or where there is little chemopreventive protection from specific components in cruciferous vegetables. These findings of gene–environment interactions emphasize the importance of cruciferous vegetable consumption and, perhaps smoking cessation, to reduce breast cancer risk, particularly among women with variant *GSTA1 *B/*B* genotypes.

GSTA1 may be particularly important in relation to breast cancer because of its interactions with sex hormones, although the evidence supporting this possibility is limited. For example, follicular maturation and luteinization increase *GSTA1* expression in the bovine and porcine ovary, which also may be induced by pituitary gonadotropins (i.e. follicle-stimulating hormone and luteinizing hormone) (25,26). In addition, 350 and 250 nM estradiol was shown to inhibit human *GSTA1* and *GSTM1* expression respectively, but not *GSTP1* and *GSTT1*, in the rat liver (27,28). *GSTA1* is present in mammary tissue, although the enzyme is expressed most abundantly in the liver (15). Furthermore, the expression of *GSTA1* does not correlate with either *GSTM1* or *GSTP1* expression in human mammary tissue (29), although *GSTM1* and *GSTP1* expressions are correlated (30), indicating an important role of GSTA1 enzyme in the breast.

Observed associations between *GSTA1* polymorphisms and breast cancer risk only among low cruciferous vegetable consumers indicate that specific components in these vegetables may be important in reduction of breast cancer risk. ITCs, contained in cruciferous vegetables, are known to induce *GSTA1* gene transcription in cultured human cells (31) and in animal models (32). Thus, it is possible that induction of *GSTA1* gene expression by ITCs among higher cruciferous vegetable consumers could override the reduced *GSTA1* gene expression due to variant *GSTA1* genotype, resulting in increased risk among women with *GSTA1* low activity genotypes and low consumption of cruciferous vegetables. Alternatively, ITCs may have anti-carcinogenic properties via independent mechanisms of *GSTA1* induction, (i.e. leading to apoptosis by activating caspases 3, 8, 9 or 12, or controlling cell cycle by modulating cell cycle regulators) (4). Thus, since ITCs could provide additional protection from DNA damage, high consumption may ameliorate risk associated with reduced expression of *GSTA1* by the polymorphism. However, the biological basis for increased risk with the *A/*A genotype with the second tertile of cruciferous vegetable consumption are unclear, and it is possible that this result is due to chance.

Although our study is the first to evaluate the association between *GSTA1* genotype and breast cancer, there have been previous reports on associations between other GST polymorphisms (i.e. *GSTM1*, *GSTP1* and *GSTT1*), ITCs and cancer risk. Spitz *et al*. (33) found that lung cancer risk was greatest among those with GST null genotypes who were low consumers of ITCs, which is consistent with our findings. They suggested that since ITCs induce GST expression, the greatest cancer risk was observed among those of a GST null genotype and low ITC intakes. Wark *et al*. (34) demonstrated that consumption of cruciferous vegetables was associated positively with GST enzyme activity among those with the *GSTM1*-positive genotype, but not those with the *GSTM1*-null genotype, suggesting that ITCs may be primarily responsible for this GST inducing capacity. Similarly, another study found that among individuals with low urinary ITC level, *GSTM1* (OR, 2.35, 95% CI, 1.02–5.41) and *GSTT1* (OR, 1.53, 95% CI, 0.68–3.44) null genotypes were associated with increased lung cancer risk. Furthermore, when the population was stratified by *GSTM1* or *GSTT1* genotypes, associations between risk and urinary ITCs were more pronounced among those with GST null genotypes (35,36). Those authors also suggest that GSTs may influence associations between risk and ITCs through their role in the metabolism and excretion of chemopreventive agents, such as ITCs. We (C.B.A.) recently investigated associations between breast cancer risk, consumption of cruciferous vegetables, and *GSTM1* and *GSTT1* genotypes in the Western New York Diet Study, and observed no significant interaction effects of *GST* genotype on breast risk, although sample size for women with genotype data available was small (5).

Tobacco smoke contains numerous carcinogens, including nitrosamines, polycyclic aromatic hydrocarbons and aromatic amines. Thus, it is possible that genotypes resulting in reduced expression of *GSTA1* are associated with increased breast cancer risk in the presence of tobacco smoke carcinogens, although a single *GST* polymorphism may not be enough to elevate risk in the absence of carcinogenic exposures. Results from several other studies are consistent with our findings. As reviewed by Rebbeck (37), several studies have shown that individuals with *GSTM1* and *GSTT1* null alleles are at increased risk of lung and bladder cancer, both of which are associated with exposure to chemical carcinogens (e.g. cigarette smoking). Zheng *et al*. (38) also reported that *GSTM1* and *GSTT1* null genotypes were associated with a 60% increased risk of breast cancer, particularly among smokers.

Although our findings on *GSTA1* variant alleles, when stratified by menopausal status, are based on relatively small numbers, the increased risk among pre-menopausal smokers could imply that the effects of *GSTA1* polymorphisms would be pronounced in a high carcinogen environment. Although it is not clear why associations between risk, *GSTA1* genotypes and smoking are more pronounced among pre-menopausal

women, it is possible that estrogens may play a role, particularly in induction of *GSTA1* expression (27). However, biochemical studies to identify and elucidate possible mechanisms are needed.

These results could also be affected by sources of bias that are common to case–control studies (e.g. recall bias) (39), or to misclassification related to genotyping. Although genotyping data is not susceptible to the problem of recall bias, diet and other interview data may be susceptible to recall biases, which may have an impact on the interaction observed. One shortcoming of the present study was that we were not able to measure biomarkers of urinary excretion of ITCs; such measurements may be less susceptible to bias resulting from dietary measurement, although they have other potential biases related to case–control status. In addition, study participants were limited to English-speaking women (>97% of Long Island residents spoke English at the time this study was undertaken) (35). If English speakers differ from non-English speakers in cruciferous vegetable consumption, smoking status and genotype frequency, results based on LIBCSP data may not be generalizable to all the women. Because other *GST* genotype data (i.e. *GSTM1* and *GSTP1*) were not available, we were unable to conduct combined analyses. However, *GSTA1* genotype may play an independent role in the breast, since the expression of *GSTA1* did not correlate with either *GSTM1* or *GSTP1* in human mammary tissue (29). Finally, since several stratified analyses were conducted, the results may be attributable to chance.

In summary, breast cancer risk was elevated among women with the *GSTA1 *B/*B* genotype who consumed lower amounts of cruciferous vegetables or who were current smokers. A significant inverse trend was observed between increasing cruciferous vegetable consumption and decreasing breast cancer risk among women with the **B/*B* genotype. Higher consumption (4+ servings/week) ameliorated the observed increased risk associated with the genotype. To our knowledge, this is the first study to evaluate *GSTA1* genotypes and breast cancer risk. It is based on data from a large population-based case–control study with adequate statistical power and in-depth interview assessments to be able to assess potential associations. Although genotype cannot be changed, consumption of diets rich in these vegetables and, perhaps avoidance of smoking, can be undertaken for breast cancer risk reduction.

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